SDS PAGE with Phastsystem

Materials

- Buffer strips
  - PhastGel SDS-PAGE 0.20 M tricine, 0.20 M Tris, 0.55% SDS,
  - Buffer Strips pH 8.1
- SDS PAGE Gel
  - %acrylamide 10-15
  - Separation range native 80-440
  - Separation range DS 15-150
  - DNA separation range base pair 100-1500
  - Stacking zone (13mm) composition 6/3
  - Separating zone (32 mm) 10-15/2

Method

1. Prepare your samples before you start positioning the gel(s). As a guide, for the 8 well applicator, a sample of 0.5 to 1mg will be sufficient. Overloading will distort the pattern of the gel running. The normal 2x loading buffer and boiling is required.
2. Ensure that the program appropriate to your sample requirements has been entered, use program 7
3. To position the gel you have chosen (having removed the protective cover), put a few drops of dH2O on the gel bed and place the gel on top with the tab (bent up for easy positioning) facing you and so that there are no air bubbles between. Gently wipe away any surplus water.
4. Put the buffer strip holder on top ensuring that it is clipped into the pegs, and put a buffer strip in at each end for each gel, running your finger along the top to ensure an even connection of buffer to gel.
5. Put the electrode bar down and gently smooth your finger across all the electrodes to ensure that the supply is evenly delivered across the buffer strip.
6. Fill the sample applicator and place it in the slot nearest you so that when it is lowered onto the gel it will go onto the stacker area.
7. Press SEP start/stop and enter the number of gels when prompted (parameters are programmed for one gel and automatically adjusted if you run two). Press DO, then the method number, then DO, and your method will run.

Staining reagents

Coomassie Blue dye

Time to result: 25–45 min, according to gel type, the longer the better. Refresh every hour